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Review

The molecular signature of oncofusion proteins in acute myeloid leukemia

Joost H.A. Martens, Henk G. Stunnenberg *

Radboud University, Department of Molecular Biology, Faculty of Science, Nijmegen Centre for Molecular Life Sciences, 6500 HB Nijmegen, The Netherlands

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ABSTRACT

Acute myeloid leukemia (AML) associated translocations often cause gene fusions that encode oncofusion proteins. Although many of the breakpoints involved in chromosomal translocations have been cloned, in most cases the role of the chimeric proteins in tumorigenesis is not elucidated. Here we will discuss the fusion proteins of the 4 most common translocations associated with AML as well as the common molecular mechanisms that these four and other fusion proteins utilize to transform progenitor cells. Intriguingly, although the individual partners within the fusion proteins represent a wide variety of cellular functions, at the molecular level many commodities can be found.

1. Acute myeloid leukemia

Acute myeloid leukemia (AML) is a quickly progressive malignant disease in which there are too many immature blood-forming cells accumulating in the bone marrow and interfering with the production of normal blood cells. AML is the most common acute leukemia affecting adults, and its incidence increases with age [1]. With approximately 1% of cancer deaths worldwide AML is a relatively rare disease. Still, its incidence is expected to increase as the population ages. The symptoms of AML are caused by replacement of normal bone marrow with leukemic cells, resulting in a drop in red blood cells, platelets, and normal white blood cells. The early signs of AML include fever, weakness and fatigue, loss of weight and appetite, and aches and pains in the bones or joints. Other signs of AML include tiny red spots in the skin, easy bruising and bleeding, frequent minor infections, and poor healing of minor cuts. As an acute leukemia, AML progresses rapidly and is typically fatal within weeks or months if left untreated. However, acute myeloid leukemia is a potentially curable disease, although only a minority of patients are cured with current therapies.

The majority of cases of AML are associated with non-random chromosomal translocations [2] that often result in gene rearrangements. A wide variety of different studies have provided evidence for the central role of gene rearrangements in the initiation of leukemia. The most important ones showed that gene rearrangements closely correlate with specific tumor phenotypes, that successful treatment is paralleled by a decrease or eradication of the disease associated chimera, that gene fusion constructs in animal models give rise to similar disorders as those seen in human neoplasms that carry the same chimera and that silencing fusion transcripts in vitro leads to the reversal of leukemogenesis, decreased proliferation and/or differentiation [3].

Many of the gene rearrangements involve a locus encoding a transcriptional activator, leading to expression of a fusion protein that retains the DNA-binding motifs of the wild-type protein. Moreover, in many instances the fusion partner is a transcriptional protein that is capable of interacting with a corepressor complex. A commonly accepted paradigm is that through aberrant recruitment of a corepressor to a locus of active transcription, the fusion protein alters expression of target genes necessary for myeloid development, thus laying the groundwork for leukemic transformation [4]. Potential targeting of this interaction has become a major focus for development of novel therapeutics. ATRA serves as a prototype: by altering corepressor interaction with the acute promyelocytic leukemia (APL) fusion protein, ATRA effectively induces remission and has become a mainstay of treatment of this previously fatal disease [5]. However, to date, APL represents both the most curable as the best studied subtype of AML, while molecular data on other fusion proteins is limited or absent. Still, the work on PML-RARα has inspired the molecular analysis of many other AML-associated oncofusion proteins, especially AML1-ETO, CBFβ-MYH11 and MLL-fusions. Together with the analysis on some less well characterized oncofusion proteins, these studies are beginning to uncover not only the unique aspects of the various oncofusion proteins but also allow a more thorough understanding of the common molecular plateau that is used to transform cells.

* Corresponding author. Fax: +31 24 3610520.
E-mail address: h.stunnenberg@ncmls.ru.nl (H.G. Stunnenberg).

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2. Oncofusion proteins associated with AML

To date, a total of 749 recurrent balanced aberrations have been identified in AML [6]. In Table 1 representatives of recurrent balanced aberrations and their corresponding fusion genes are listed. The frequencies of the four most common translocations are between 3% and 10% while for others the prevalence is significantly smaller. Here we will first discuss the four most prevalent oncofusion proteins: PML-RARα, AML1-ETO, CBFβ-MYH11 and MLL-fusions after which we will discuss the common molecular characteristics of these and other AML related oncofusion proteins.

3. Common AML-associated translocations

3.1. t(15;17), PML-RARα

The t(15;17) translocation is found in approximately 95% of acute promyelocytic leukemias (APLs), a specific subtype of AML. The translocation results in the expression of the PML-RARα oncofusion gene in hematopoietic myeloid cells [7,8]. The PML-RARα oncofusion protein acts as a transcriptional repressor that interferes with gene expression programs involved in differentiation, apoptosis and self-renewal [9].

At the molecular level PML-RARα behaves as an aberrant RAR. In absence of all-trans retinoic acid (ATRA), RARα interacts with RXR, itself a nuclear receptor, and binds to DNA. The normal RARα/RXR heterodimer recruits corepressor complexes and represses transcription of its target genes. A conformational change caused by binding of ATRA at physiological concentrations triggers the dissociation of the corepressors and promotes the recruitment of coactivators. In contrast, PML-RARα acts as a constitutive repressor that is insensitive to physiological concentrations of ATRA [10–12]. Under physiological concentrations of ATRA, PML-RARα complexes bind RXR and form an oligomeric complex that is essential for its oncogenic potential as it facilitates binding to widely spaced direct repeats [13–15] and has been shown to be a critical determinant for the transforming potential of PML-RARα complexes [16,17]. To overcome the transforming potential of PML-RARα, human APL patients are treated during the early phase of the disease with pharmacological doses of ATRA and/or As_2O_3 [10,18]. This treatment has been shown to degrade PML-RARα and to dissociate HDACs creating a hyperacetylated chromatin state [14,19,20].

3.2. t(8;21), AML1-ETO

Approximately 10% of AML cases carry the t(8;21) translocation, which involves the *AML1* (*RUNX1*) and *ETO* genes, and express the resulting AML1-ETO fusion protein. AML1 is a DNA-binding transcription factor crucial for hematopoietic differentiation [21,22] while ETO is a protein harbouring transcriptional repressor activities [23]. The fusion protein AML1-ETO is suggested to function as a transcriptional repressor by recruiting NCoR/SMRT/HDAC complexes to DNA through its ETO moiety [23]. Moreover, it has been shown that AML1-ETO blocks AML1-dependent transactivation in various promoter reporter assays, suggesting it may function as a dominant negative regulator of wild-type AML1 [24–26]. AML1-ETO was recently hypothesized to target DNA through E-box motifs as a result of physical interactions with transcription factors of the E-protein family, in particular HEB/TCF12 [27,28].

3.3. Inv(16), CBFβ-MYH11

Inv(16) is found in approximately 8% of acute myeloid leukemia (AML) cases. Inv(16) fuses the first 165 amino acids of core binding factor β (CBFβ) to the C-terminal coiled-coil region of a smooth muscle myosin heavy chain (*MYH11*) [29]. CBFβ interacts with the AML1 transcription factor to increase the affinity of AML1 for DNA [30–32] and to stimulate the ability of AML1 to either activate or repress transcription [33,34]. In contrast, the CBFβ-MYH11 fusion protein is suggested to cooperate with AML1 to repress transcription [35]. AML1 binds the mSin3A and Groucho corepressors, and the inv(16) fusion protein can form a trimeric complex with AML1 and mSin3A [35]. In addition, the C terminus of the CBFβ-MYH11 fusion protein is required for repression [35], suggesting that the fusion protein may cooperate with AML1 to recruit corepressors. Indeed, the C-terminal MYH11 portion of inv(16) is both necessary and sufficient for transcriptional repression and is sufficient for association with SIN3A and HDAC8 [36].

3.4. 11q23, MLL rearrangements

MLL is implicated in at least 10% of acute leukemias (AL) of various types: acute lymphoblastic leukemias (ALL), acute myeloid leukemias (AML), biphenotypic ALs, treatment related leukemias and infant leukemias. In general the prognosis is poor for AML patients harbouring MLL translocations [37]. In these leukemias, the MLL protein fuses to one of >50 identified partner genes, resulting in a MLL-fusion protein that acts as a potent oncogene [38]. The amino-terminal portion of MLL serves as a targeting unit to direct MLL oncoprotein complexes to their target loci through DNA-binding [39,40] whereas the fusion partner portion serves as an effecter unit that causes sustained transactivation [41]. While extensive gene expression signatures have been determined for primary human leukemia samples [42–47], the direct genomic targets of MLL-fusion proteins in AML remain largely unknown, while only one report described the global role of MLL-AF4 fusion in ALL [48]. Preliminary results in ALL suggest that MLL-fusion proteins produce gross defects in chromatin structure through alteration of the distribution of histone modification associated with the transcription

Table 1
AML-associated oncofusion proteins.

Translocation	Prognosis	FAB	Oncofusion-protein	Occurence
t(8;21)	Favorable	M2	AML1-ETO	10% of AML
t(15;17)	Favorable	M3	PML-RARα	10% of AML
inv(16)	Favorable	M4	CBFβ-MYH11	5% of AML
der(11q23)	Variable	M4/M5	MLL-fusions	4% of AML
t(9;22)	Adverse	M1/M2	BCR-ABL1	2% of AML
t(6;9)	Adverse	M2/M4	DEK-CAN	<1% of AML
t(1;22)	Intermediate	M7	OTT-MAL	<1% of AML
t(8;16)	Adverse	M4/M5	MOZ-CBP	<1% of AML
t(7;11)	Intermediate	M2/M4	NUP98-HOXA9	<1% of AML
t(12;22)	Variable	M4/M7	MN1-TEL	<1% of AML
inv(3)	Adverse	M1/M2/M4/M6/M7	RPN1-EVI1	<1% of AML
t(16;21)	Adverse	M1/M2/M4/M5/M7	FUS-ERG	<1% of AML

start site (H3K4me3) and gene bodies (H3K79me2) of transcribed genes, but whether this is also the case in AML remains elusive.

4. Less prevalent translocations

The remaining balanced aberrations are uncommon; together constituting 6% of all AML cases. Therefore, in total approximately 35% of AMLs are characterized by translocations. Although the importance of these gene rearrangements in the onset of leukemogenesis is evident, the individual protein partners within the fusion proteins represent a wide variety of cellular functions. Still, the gene fusions all result in uncontrolled proliferation of progenitor cells. Hence, it can be expected that the various oncofusion proteins utilize common molecular mechanisms to transform cells. Indeed, as discussed below, a close examination at the actions of the individual oncofusion proteins reveals many commodities at multiple levels such as transcriptional regulation, cofactor involvement, chromatin modification and pathway usage (Fig. 1).

5. Disruption of transcription programs

Disruption of existing transcription profiles is a hallmark of AMLs harbouring chromosomal translocations. This disruption can happen at multiple levels. The TF itself can be involved in the translocation or the translocation results in an aberrantly expressed coactivator. Either way, it is generally assumed that the resulting fusion protein targets key hematopoietic regulators and thereby disrupts the normal differentiation program (Fig. 2).

5.1. Aberrant TFs

AML1-ETO is the hallmark example of an aberrantly regulated transcription factor in AML. AML1 is a DNA-binding transcription factor required for hematopoiesis [21,22], while ETO is a corepressor molecule [23]. The translocation fuses the N-terminal DNA-binding domain of AML1 to almost all of ETO resulting in the localization of the transcriptional repressor functions of ETO to AML1 target genes. Another transcription factor, CBFβ, interacts with AML1 to increase the affinity of AML1 for DNA [30–32]. As discussed above, also this transcription factor can be involved in a chromosomal translocation and the resulting CBFβ-MYH11 fusion protein is suggested to cooperate with AML1 to repress transcription [35].

Another group of translocations fuse the NUP98 protein to several homeodomain-containing transcription factors [49]. NUP98-HOXA9 is the prototype of this group and generally these fusion proteins are thought to act as aberrant transcription factors that induce proliferation and block differentiation of hematopoietic precursors [50]. Similar as for NUP98, fusion of the MLL protein with several partner genes, results in oncofusion proteins that act as potent oncogenes [38]. In these fusions, the amino-terminal portion of MLL serves as a targeting unit to direct MLL oncoprotein complexes to their target loci through DNA-binding [39,40] whereas the fusion partner portion serves as an effecter unit that causes sustained transactivation of target genes [41]. RPN1-EVI1 represents a fifth example of transcription factor involvement in oncofusion proteins. Fusion constructs of EVI1 are thought to result in activation of the expression of EVI1, thereby interfering with the cellular mechanisms that control EVI1 expression. EVI1 is a nuclear transcription factor involved in many signaling pathways for both corepression and coactivation of cell cycle genes. Although the underlying molecular mechanisms have not been elucidated, in vitro experiments suggest that EVI1 expression prevents the terminal differentiation of bone marrow progenitor cells to granulocytes and erythroid cells [51].

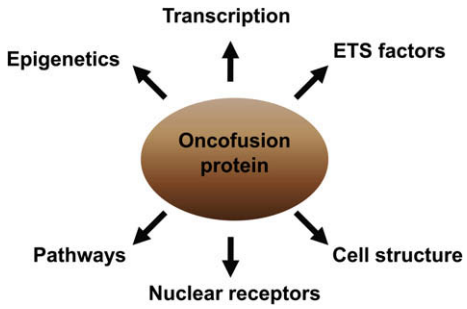

Fig. 1. Common molecular targets of oncofusion proteins.

5.2. Coactivators

Apart from misregulation at the level of DNA-binding transcription factors, several AMLs display gene rearrangements that result in aberrant expression of transcriptional coactivators. For example, one of the common targets of chromosomal translocations is the histone acetyltransferase MOZ. *MOZ* was first identified as a gene involved in the translocation t(8;16) resulting in the *MOZ–CBP* fusion gene. The *MOZ–p300*, *MOZ–TIF2* and *MOZ–NcoA3* fusion genes were later identified in AMLs harboring t(8;22), inv(8) and t(8;20), respectively [52]. The non-fused MOZ has been shown to modulate gene transcription through activation of transcription factor complexes [53]. In its turn CBP and p300 are major HATs that function as coactivators for various transcription factors, while TIF2 (NcoA2/GRIP1) and NcoA3 (TRAM-1/RAC3/pCIP/AIB-1) are adaptor proteins that combine nuclear receptors with CBP. It has been suggested that the fusion of MOZ with the above proteins alters its coactivator capacities as compared to the non-fused protein. These alterations in the coactivation potential of the fused protein have been hypothesized to be the main mechanism of leukemogenesis in these AML varieties [52,53].

As MOZ, MN1, which is involved in the t(12;22) translocation, acts as a transcriptional coactivator [54,55]. t(12;22) fuses almost all the coding sequence of *MN1* to two-thirds of the coding sequence of *TEL* (*ETV6*). MN1-TEL is thought to act as a novel transcription factor causing transcriptional deregulation of genes normally repressed by TEL [56]. In addition, through its MN1 moiety, MN1-TEL has been shown to repress RARα/RXR-mediated transcription [57].

Finally, MAL, one of the fusion partners in the OTT-MAL oncofusion protein, has been described as a potent transcriptional coactivator of the myocardin-related transcription factor (MRTF) family, which is implicated in gene expression by serum response factor (SRF) [58]. Again fusion of the transcription coactivator alters its normal function and is suggested to result in the constitutive activation of SRF target gene expression [59,60].

5.3. Hematopoietic master regulators

Aberrant regulation of proteins that determine hematopoietic cell fates is central to the onset of AML. Indeed, many of the above described fusion proteins are directly involved in altering the transcriptional program of key regulators of hematopoiesis. The best example is the t(8;21) chromosomal aberrations affecting the key hematopoietic regulator AML1, while the CBFβ-MYH11 oncofusion protein can be expected to exert similar effects on the hematopoietic differentiation programs regulated by AML1, due to its interaction with this protein [30–32]. Similarly, MOZ has been shown to modulate gene transcription through activation of the AML1 and SPI1 transcription factor complexes. Therefore, inhibition of key hematopoietic regulator mediated transcription by MOZ-fusion

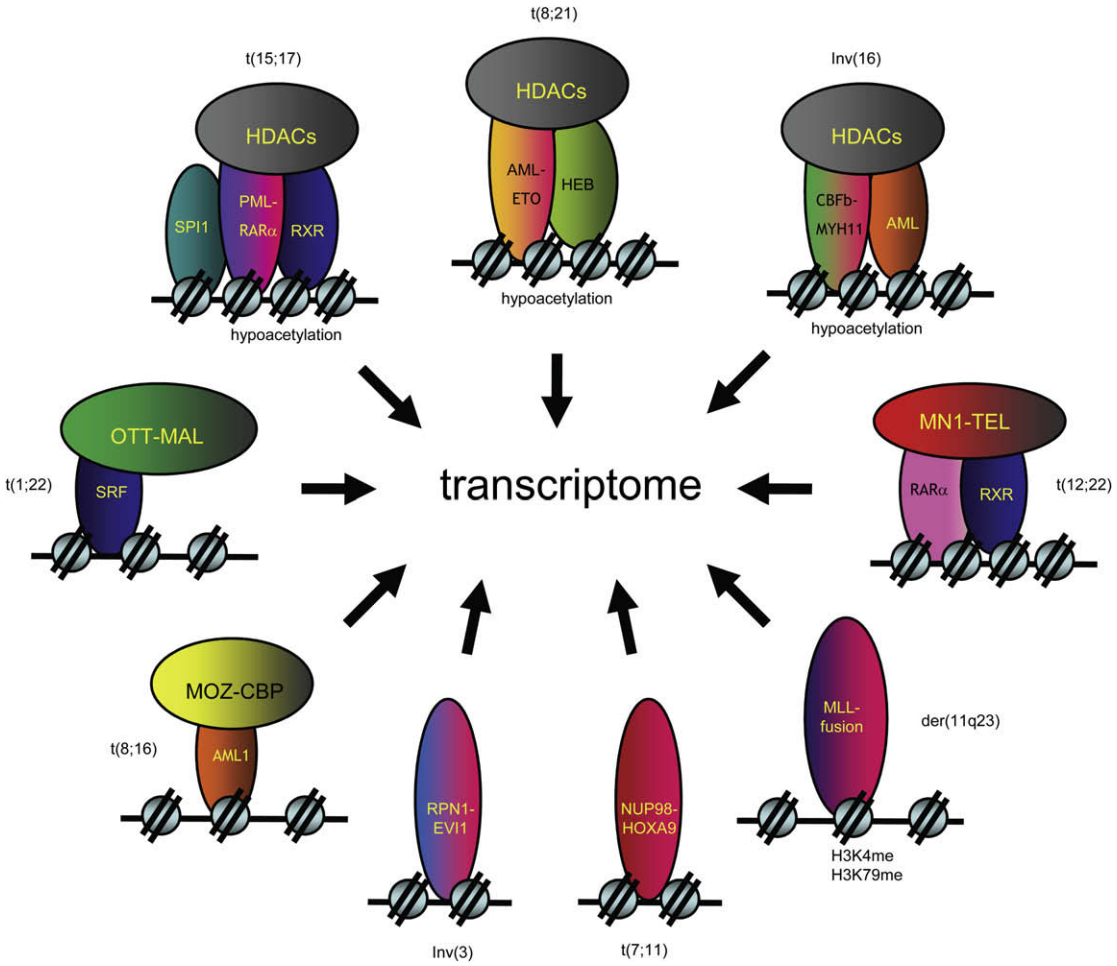

Fig. 2. Overview of DNA-binding oncofusion proteins.

proteins has been hypothesized to be the main mechanism of leukemogenesis in this AML variety [52,53].

Apart from aberrant regulation of the key hematopoietic master regulator protein itself, oncofusion proteins might also affect these proteins through regulation of their expression. This is exemplified by the recent analysis of genome-wide binding sites of PML-RARα [14] that suggested regulation of several key hematopoietic master regulator genes, such as *GFI1*, *AML1* and *SPI1* (Fig. 3) by this protein.

6. Nuclear receptor signalling

Nuclear receptors are key regulators of cell growth and differentiation, homeostasis and development and represent a large family of ligand-dependent transcription factors [61]. In addition, nuclear receptors represent key targets for treatment, exemplified by the great number of studies dedicated to examining the effects of nuclear receptor ligands on cancer cells. The hallmark nuclear receptor associated with AML is RARα which is directly misregulated through chromosome translocations involving chromosome 17. Indeed RARα-fusions such as PML-RARα and PLZF-RARα are hallmark proteins of AML FAB M3. Apart from affecting RARα these translocation also target RXR, another nuclear receptor, through direct interaction with this protein [14,16,17]. RARα has also been suggested to interact with AML1-ETO in regulating expression of the RARβ gene [62], although it is currently unclear whether this interplay can be extended to other RARα regulated genes. In addition, recent findings indicate that another oncofusion protein, MN1-TEL, represses RARα/RXR-mediated transcription through its MN1 moiety [57]. Together these results identify the retinoic acid signalling pathway as a major target of oncofusion proteins.

In addition to the nuclear receptor RAR, it is not unlikely that oncofusion proteins might also aberrantly regulate other nuclear receptors. Indeed, it has been suggested that CAN (NUP214), one of the fusion partners of DEK-CAN interacts and modulates the function of the vitamin D receptor (VDR) [63], thereby implying a role for DEK-CAN in disturbing VDR regulated pathways.

7. Structural organisation

Often one of the partners in oncofusion proteins represent a structural protein. Examples of these include the PML body associated PML protein in PML-RARα, and the nuclear pore complex proteins NUP214 (CAN) and NUP98 in DEK-CAN and NUP98-HOXA9, respectively. At the molecular level these structural components are suggested to be vital for the oligomerization properties of these oncofusion proteins. In this case oligomerization is suggested to induce an altered interaction with transcriptional coregulators and thereby representing an alternative mechanism of oncogenic activation [64]. Another possibility is that the fusion disrupts the original structure that contained the non-fused protein. This is exemplified by the disruption of PML bodies in PML-RARα expressing APL cells. Similarly, expression of the nucleoporin-fusion proteins might result in alteration in nuclear pore complexes and in aberrant nucleocytoplasmic transport. An intriguing possibility is

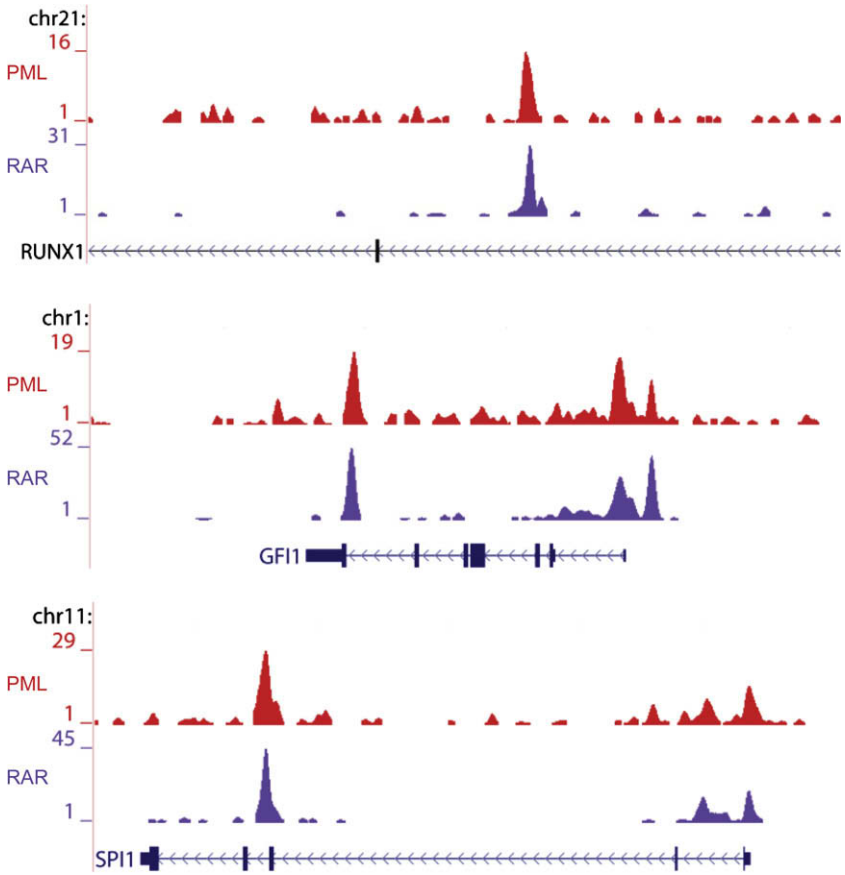

Fig. 3. PML-RARα and RXR colocalization to genomic regions in NB4 cells. Overview of the *AML1* (*RUNX1*), *GFI1* and *SPI1* PML-RARα/RXR binding sites. In red PML ChIP-seq data is plotted, in purple RARα data.

that these alterations in specific cellular structures are reflected in the morphology of the cell. Oncofusion proteins are associated with different FAB types (Table 1), i.e. different morphologies. Although these can be due to the differentiation step in which the fusion gene functions or the oncofusion protein induced gene program, the structural aberration might also be a consequence of the gene fusion itself.

8. ETS factors

The *SPI1* (*PU.1*) gene encodes an ETS-domain transcription factor that is a master regulator of gene expression during myeloid cell development. SPI1 has recently been identified both as a partner within the PML-RARα complex [65] as well as a factor that is regulated at the transcriptional level by the PML-RARα protein itself [14]. Moreover, SPI1 was identified as a downstream target of AML1 [66], opening the possibility that it is also transcriptionally regulated by AML1-ETO in t(8;21) cells. In addition to SPI1, other ETS factors, such as TEL and ERG have been associated with AML. TEL is directly targeted in a gene rearrangement with MN1 while ERG has been identified in a chromosomal translocation involving FUS. While the molecular mechanisms of FUS-ERG mediated transformation are unknown, MN1-TEL is thought to act as a novel transcription factor causing transcription deregulation of genes normally repressed by TEL [56]. Moreover, as TEL normally binds FLI1, aberrant regulation of FLI1 might also be involved in causing the proliferative state in the MN1-TEL expressing AMLs. Together with SPI1, these examples suggest an important role of ETS factors in leukemogenesis.

9. Epigenetics

9.1. Acetylation

Throughout the years many studies have been dedicated to the identification of epigenetic modifications associated with oncofusion protein binding. This work was largely inspired by PML-RARα which was the first protein complex in which, with the identification of HDAC association, an epigenetic factor was found. These studies were later followed by suggestions of PML-RARα mediated recruitment of DNMTs, EZH2 and SUV39H and the subsequent establishment of a repressive chromatin environment with low acetylation, high DNA methylation, high H3K27me3 and high H3K9me3 at PML-RARα binding sites. However, these studies were largely focussed on the promoter of the RARβ gene and a global analysis showed that for most PML-RARα target sites many of the previous suggested epigenetic modifications were not present and that essentially only HDACs were recruited [14], for example to the *CCL2* gene (Fig. 4). Analysis of other AML-associated oncofusion proteins seem to confirm the intimate connection between these proteins and the acetylome. AML1-ETO is suggested to function as a transcriptional repressor by recruiting NCoR/SMRT/HDAC complexes to DNA through its ETO moiety [23], while the transcriptional repression mechanisms of the CBFβ-MYH11 fusion protein are also suggested to be mediated through cooperation of the fusion protein with AML1 and recruitment of HDAC containing corepressor complexes [35]. Changes in the acetylome can also be expected in AMLs that harbour translocations that affect particular coactivator proteins. The hallmark examples of these include

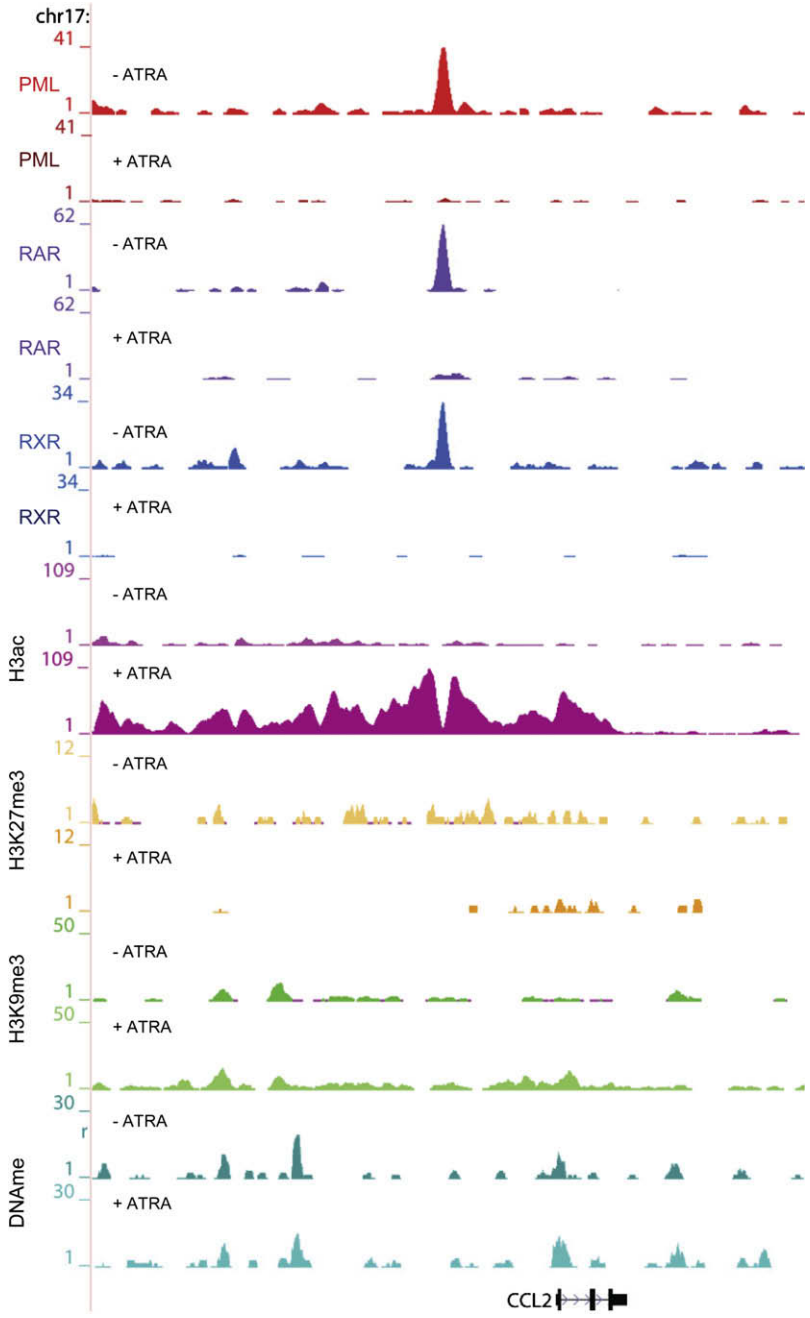

Fig. 4. ATRA induces epigenetic changes at the *CCL2* PML-RARα/RXR binding region in NB4 cells. In red the PML, in purple the RARα and in blue the RXR ChIP-seq data is plotted for proliferating and 24 h ATRA treated NB4 cells. For H3K9K14ac (pink), H3K27me3 (yellow), H3K9me3 (green) and DNAme (turquoise) the data is plotted both for proliferating and for 24 h (H3K9K14ac and H3K27me3) or 48 h (DNAme) ATRA treated NB4 cells.

the translocations involving MOZ. At the molecular level, translocation t(8;16) fuses MOZ and CBP genes while t(8;22) fuses MOZ and p300, all three encoding proteins with histone acetyltransferase activity [52]. Similarly, the OTT coactivator which is part of the fusion OTT-MAL, contains a highly conserved SPOC domain which is suggested to interact with SMRT and NCoR corepressor complexes and thereby recruit HDAC activities [60].

9.2. Other chromatin alterations

Apart from changes in the acetylome, numerous other chromatin changes have been suggested to be associated with specific oncofusion proteins. For example, MLL-fusions have been described to result in aberrant recruitment of enzymes that can mediate histone H3 lysine 4 and 79 methylation [48,67] and these two chromatin marks are therefore expected to be hallmarks of the MLL-fusion epigenome. Chromatin organisation is also likely to be affected in the AML subtypes harboring the t(11;20) or the t(6;9) translocation that express the NUP98-TOP1 and DEK-CAN oncofusion proteins, respectively, as both Topoisomerase 1 (TOP1) and DEK have been recognized for their key roles in relaxing supercoiled DNA [68,69]. Moreover, a recent study identified DEK as a histone chaperone [70]. Therefore, aberrant regulation of these two proteins can be expected to have a significant impact on chromatin integrity.

For DNA methylation, PML-RARα mediated recruitment of DNA methyltransferases to the RARβ promoter has long been used as the prime example of the interplay between oncogenes and this

epigenetic mark. However, recent global analysis could not extend the recruitment of DNA methyltransferases to all PML-RARα binding sites [14]. Still, recent large-scale DNA methylation profiling revealed the existence of distinct DNA methylation patterns in AML and identified novel, biologically and clinically relevant defined AML subgroups [71]. These findings suggest that although some epigenetic alterations might not be directly related to oncofusion protein binding, the indirectly induced epigenetic state might still be highly correlative with the expressed fusion gene.

10. Pathways

In many instances one of the partners in a gene rearrangement codes for a transcriptional protein. As a consequence, AML-associated fusion proteins often function as aberrant transcriptional regulators. The resulting expression profiles can vary significantly and can even be used to classify AMLs harboring different gene rearrangements [72]. Despite these variations in gene expression all AML-associated fusion proteins interfere with the process of myeloid differentiation, suggesting that ultimately their unique molecular properties converge into common molecular mechanisms to transform cells. A plausible explanation on how this can be achieved would be that ultimately similar pathways are affected by the fusion proteins. Therefore it is essential to elucidate the downstream pathways and biological processes where oncofusion protein-regulated genes are contributing. However, thus far these analysis have been limited and only one study tried to identify the common pathways of several oncofusion proteins [73]. This transcription analysis of AML1-ETO, PML-RARα and PLZF-RARα expressing cells revealed aberrant regulation of pathways involved in signaling, apoptosis and cell structure. Other, non-comparative, studies involving single oncofusion proteins seem to confirm the role of these proteins in signaling and apoptosis. For example BCR-ABL activates the Ras signal transduction pathway via it's linkage to son-of-sevenless (SOS), a Ras activator and has been shown to inhibit apoptosis. Similarly, EVI1, a fusion partner in RPN1-EVI1, has been shown to be involved in the downstream signaling pathway of transforming growth factor beta (TGF-β) and in inhibiting JNK dependent activation of key transcription factors for the apoptotic response. Still, it remains to be determined whether the apoptotic and the various signaling pathways are also targets of other oncofusion proteins.

11. Outlook

Although many of the breakpoints involved in specific chromosomal translocations have been cloned and novel ones are still being discovered, in most cases the molecular mechanisms and the central players leading to tumorigenesis are not elucidated. Often, the chromosomal translocation leads to the expression of fusion genes that encode chimeric proteins that can drive oncogenic transformation. It is becoming exceedingly clear that a detailed knowledge of the molecular pathways influenced by the expression of these oncofusion proteins has an enormous potential and will lay the basis for diagnosis, prognosis and drug therapy development. However, the progress in elucidating the actions of oncofusion proteins has been hampered by the lack of tools to perform a comprehensive analysis. This limitation has recently been overcome by the development of several high-throughput DNA sequencing techniques that allow large-scale identification of chromosomal aberrations, gene fusions, (oncofusion) protein binding, gene expression and epigenetic modifications. Therefore, it is now within our reach to perform a detailed characterization of the genomic targets of oncofusion protein as well as the effects of these proteins on gene transcription and the epigenetic state. Apart from providing novel insights into the etiology of cancer, a detailed understanding of the general mechanisms associated with cancer onset is likely to provide a rationale for therapy design and epigenetic biomarker development.

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